Federal Democratic Republic of Ethiopia

EDICT OF GOVERNMENT

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ET ISO 16931 (2012) (English): Animal vegetables fats and oils - Determination of polymerized triacylglycerols by high-performance size-exclusion chromatography (HPSEC)

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ETHIOPIAN
STANDARD

ES ISO 16931:2012

First edition

Animal and vegetable fats and oils-Determination of polymerized triacylglycerol by high-performance size exclusion chromatography(HPSEC)

(Identical with ISO 16931:2009)

ICS: 67.200.10

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Foreword

This Ethiopian Standard has been prepared under the direction of the Technical Committee for Animal and vegetables fats and oils (TC 23) and published by the Ethiopian Standards Agency (ESA).

The standard is identical with ISO 16931:2009, Animal and vegetable fats and oils - Determination of polymerized triacylglycerols by high-performance size-exclusion chromatography (hpsec), published by International Organization for Standardization (ISO).

For the purpose of this Ethiopian Standard, the adopted text shall be modified as follows.

- The phrase “International Standard” shall be read as “Ethiopian Standard”; and
- A full stop (.) shall substitute comma (,) as decimal marker.

Animal and vegetable fats and oils — Determination of polymerized triacylglycerols by high-performance size-exclusion chromatography (HPSEC)

1 Scope

This International Standard specifies a method using high-performance size-exclusion chromatography (HPSEC) to determine the contents, as mass fractions, of polymerized triacylglycerols (PTAGs) in oils and fats which contain at least 3 % (from peak areas) of these polymers. PTAGs (strictly speaking dimeric and oligomeric triacylglycerols) are formed during the heating of fats and oils, and thus, the method serves to assess the thermal deterioration of frying fats after use.

This method is applicable to frying fats and fats and oils that have been thermally treated, provided that the content of PTAGs is at least 3 %. It can also be applied to the determination of polymers in fats for animal feedstuffs, although in this case, the extraction method used can have an influence on the result.

NOTE For further details, see ISO 6492[4].

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 661, *Animal and vegetable fats and oils — Preparation of test sample*

3 Terms and definitions

For the purposes of this International Standard, the following terms and definitions apply.

3.1
polymerized triacylglycerols
PTAGs

constituents of heated fats and oils that are determined by HPSEC under the conditions specified in this International Standard

NOTE The content of PTAGs is expressed as a percentage mass fraction, in grams per 100 g, of all peaks from eluted polymerized and mono-, di-, and triacylglycerols (PTAGs, MAGs, DAGs, and TAGs).

4 Principle

The sample is homogeneously mixed with tetrahydrofuran (THF) and PTAGs separated by gel permeation chromatography according to molecular size. The compounds are detected by means of a refractive index detector.

NOTE For better resolution, two columns in series (2 × 300 mm) are used.

5 Reagents

Use only reagents of recognized analytical grade.

WARNING — Attention is drawn to the regulations which specify the handling of hazardous substances. Technical, organizational and personal safety measures shall be followed.

5.1 Tetrahydrofuran (THF), for HPLC, possibly stabilized with butylhydroxytoluene (BHT), degassed, free of water.

Ensure that the THF used as a diluent for the sample has the same water content as the eluent; otherwise a negative peak can be observed.

5.2 Toluene, for HPLC.

5.3 Extra virgin olive oil, as standard reference: add 100 mg to 300 mg of oil to 10 ml THF and homogenize the mixture.

NOTE Extra virgin olive oil does not contain PTAGs and can be used to determine the retention time of the monomeric TAGs.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Solvent reservoir, capacity 500 ml to 1 000 ml, with a polytetrafluoroethylene mobile-phase line filter.

6.2 HPLC pump, pulseless, with a volume flow rate of 0,5 ml/min to 1,5 ml/min.

6.3 Injection valve, with a 20 µl loop and a suitable syringe with a volume of 50 µl to 100 µl, or a suitable **autosampler**.

6.4 Stainless-steel columns, length 2 × 300 mm, internal diameter 7,5 mm to 7,8 mm, packed with spheres, diameter 5 µm, of high-performance styrene-divinylbenzene co-polymer gel; of pore size 10 nm or 50 nm[1].

NOTE The chromatograms in Annex A show the required resolution with two columns.

The use of a guard column is recommended. The efficiency of the column, determined as the number, n, of theoretical plates for monomeric TAGs, should be at least 6 000.

Maintain the temperature of the column between 30 °C and 35 °C by means of a control device.

If necessary, the column can be stored in toluene (5.2), although experience has shown that it is better to keep the system running with THF.

6.5 Detector, temperature-controlled refractive index detector.

The ideal temperature for the detector is just above that of the column (30 °C to 35 °C).

6.6 Chromatography data system (CDS), to allow display and accurate quantification of the peak areas.

6.7 Single-use syringes, capacity 1 ml.

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[1] 1 nm = 10 Å

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6.8 **Nylon filters**, pore size 0,45 µm2).

6.9 **HPLC syringe**, capacity 50 µl to 100 µl.

7 Sampling

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transportation or storage.

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 5555[1].

8 Preparation of test sample

The test sample shall be prepared in accordance with ISO 661.

9 Procedure

9.1 Starting up the HPLC equipment

It is advisable to follow carefully the manufacturers' recommendations. Switch on the system and pump THF at a volume flow rate of between 0,5 ml/min and 1 ml/min to purge the whole system up to the injection valve. Connect the column to the injection valve and wash it with about 30 ml of THF. Connect the column to the sample cell of the detector. Fill the reference cell with THF. Adjust the mobile-phase flow to between 0,5 ml/min and 1,0 ml/min. Wait until a convenient stabilization of the system (no appreciable deviation of the baseline) is obtained.

If the column recommended in this subclause is used, an acceptable stabilization of the system should be obtained in about 15 min. With other column packings, the stabilization of the system may be more difficult. For example, changing the mobile phase should be done stepwise from toluene to THF, with different mixtures, each containing 25 % more THF. Acceptable stabilization is normally obtained in about 12 h.

The following conditions have been found to be suitable:

Column:	PLgel3) 10 nm, 2 × 300 mm × 7,6 mm, 5 µm;
Eluent:	THF;
Flow:	0,8 ml/min;
Column oven:	35 °C;
Detector:	RI set at 35 °C;
Injection volume:	20 µl.

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2) Nalgene 4 mm Syringe Filter is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product. Equivalent products may be used if they can be shown to lead to the same results.

3) PLgel is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product. Equivalent products may be used if they can be shown to lead to the same results.

9.2 Preparation of test portion and analysis

Add about 100 mg to 300 mg of the test sample (Clause 8) to 10 ml of THF and homogenize. If necessary, filter through a nylon filter (6.8). Inject, with the HPLC syringe (6.9), 20 µl to 40 µl of that solution.

10 Expression of results

10.1 Qualitative analysis

The chromatographic pattern of the determination shows a main peak representative of monomeric TAGs (relative molecular mass about 900) and one or several smaller peaks with shorter retention times representative of PTAGs (dimers and upper oligomers).

Under suitable conditions, TAGs and PTAGs can be separated with good resolution (Figures A.1 and A.2) even at low levels of PTAGs.

However, in some cases (which seem to be connected to complex degradation phenomena), the peak pattern preceding the triacylglycerol peaks can be less clear with consequent difficulties for the calculations (Figure A.2).

10.2 Quantitative analysis

Calculation is carried out by the internal normalization method, assuming that all components of the sample which are eluted have the same response coefficient. It is important to have a straight baseline.

The mass fraction, in grams per 100 g of the PTAGs, w_{PTAG}, is calculated using the following equation:

$$w_{\text{PTAG}} = \frac{\sum A_{\text{PTAG}} \times 100}{\sum A_{\text{tot}}}$$

where

A_{PTAG} is the sum of the peak areas of all PTAGs;

A_{tot} is the sum of the peak areas of all acylglycerols (PTAGs, TAGs, DAGs, and MAGs).

Express the results to one decimal place.

11 Precision

11.1 Interlaboratory tests

Details of interlaboratory tests on the precision of the method are summarized in Annex B. The values derived from these interlaboratory tests may not be applicable to concentration ranges and matrices other than those given.

11.2 Repeatability

When the values of two independent test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, lie within the range of the mean values given in Table B.1, the absolute difference between the two test results obtained shall, in not more than 5 % of cases, be greater than the repeatability limit, r, which can generally be deduced by linear interpolation from the values in Table B.1.

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11.3 Reproducibility

When the values of two independent test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, lie within the range of the values given in Table B.1, the absolute difference between the two test results obtained shall, in not more than 5 % of cases, be greater than the reproducibility limit, R, which can generally be deduced by linear interpolation from the values in Table B.1.

12 Test report

The test report shall contain at least the following information:

a) all information necessary for the complete identification of the sample;

b) the sampling method used, if known;

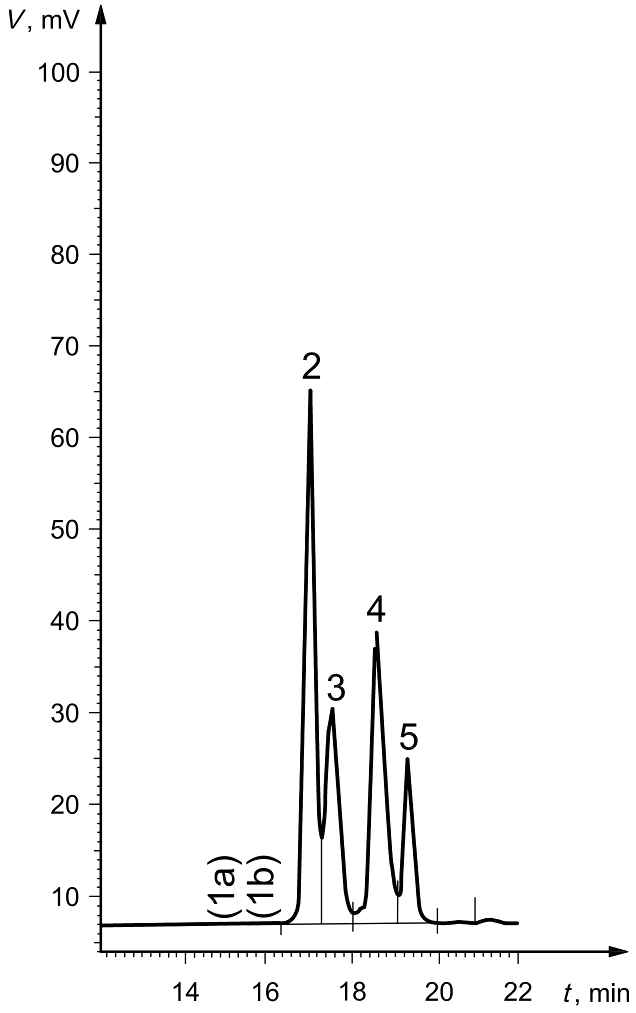
c) the test method used, with reference to this International Standard;

d) all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

e) the test result(s) obtained or, if the repeatability has been checked, the final result obtained.

Annex A
(informative)

Chromatograms



Key

1a/1b	poly-/oligomeric TAGs
1b	dimeric TAGs
2	TAGs
3	DAGs
4	MAGs
5	fatty acids
t	time
V	response

NOTE Conditions are given in 9.1.

Figure A.1 — HPSEC of a test mixture of TAGs, DAGs, MAGs and free fatty acids

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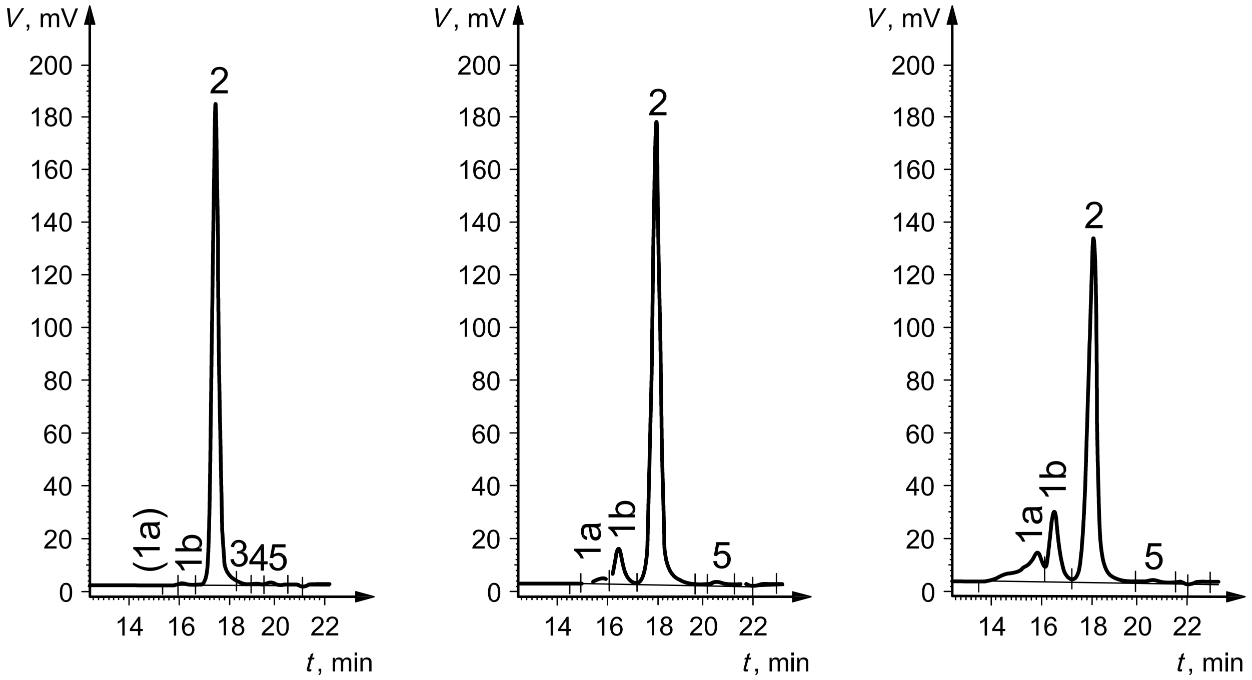

a) ~ 1 % PTAG

b) 10 % PTAG

c) 30 % PTAG

Key

1a/1b	poly-/oligomeric TAGs
1b	dimeric TAGs
2	TAGs
3	DAGs
4	MAGs
5	fatty acids
t	time
V	response

NOTE 1 Conditions are given in 9.1.

NOTE 2 The elution order is defined by the molecular size and relative molecular mass. During frying, no DAGs are formed. However, the peak from DAGs increases due to oxidation resulting from thermal degradation of TAGs to TAGs with two C_7 or C_8 fragments and a C_{18} fatty acid. The relative molecular mass is equal to that of DAGs.

Figure A.2 — HPSEC of TAGs and PTAGs: chromatograms of frying oils with different mass fractions of PTAGs

Annex B
(informative)

Results of interlaboratory tests

An interlaboratory tests carried out at an international level in 2007 by DIN and DGF, in which 15 laboratories participated, each obtaining two test results for each sample, gave the statistical results (evaluated in accordance with ISO 5725-1:1994[2] and ISO 5725-2:1994[3]) summarized in Table B.1.

Table B.1 — Results of interlaboratory tests

Samples of used frying oils	**A**	**B**	**C**	**D**	**E**
Number of laboratories	15	15	15	15	15
Number of laboratories retained after eliminating outliers	14	15	13	15	14
Number of accepted results	28	30	26	30	28
Mean, $\bar{w}_{\text{PTAG}}$, %	**5,23**	**11,95**	**12,81**	**15,78**	**20,42**
Repeatability standard deviation, s_r	0,10	0,12	0,11	0,13	0,14
Repeatability coefficient of variation, CV(r), %	1,9	1,0	0,8	0,8	0,7
Repeatability limit, r (2,8 s_r)	**0,28**	**0,32**	**0,30**	**0,37**	**0,39**
Reproducibility standard deviation, s_R	0,22	0,20	0,19	0,26	0,15
Reproducibility coefficient of variation, CV(R), %	4,1	1,7	1,4	1,6	0,7
Reproducibility limit, R (2,8 s_R)	**0,61**	**0,57**	**0,52**	**0,72**	**0,42**
NOTE Results and statistics are expressed in grams per 100 grams (percentage mass fractions).					

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Bibliography

[1] ISO 5555, *Animal and vegetable fats and oils — Sampling*

[2] ISO 5725-1:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

[3] ISO 5725-2:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

[4] ISO 6492, *Animal feeding stuffs — Determination of fat content*

[5] IUPAC Method 2.508, Determination of polymerized triglycerides in oils and fats by high performance liquid chromatography. *Pure Appl. Chem.* 1991, **63**, pp. 1168-1171. Available (2008-10-16) at: http://www.iupac.org/publications/pac/1991/pdf/6308x1163.pdf

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Ethiopian Standards Agency

Organization and Objectives

The Ethiopian Standards Agency (ESA) is the national standards body of Ethiopia established in 2010 based on regulation No. 193/2010.ESA is established due to the restructuring of Quality and Standards Authority of Ethiopia (QSAE) which was established in 1970.

ESA's objectives are:-

- Develop Ethiopian standards and establish a system that enable to check weather goods and services are in compliance with the required standards,
- Facilitate the country's technology transfer through the use of standards,
- Develop national standards for local products and services so as to make them competitive in the international market.

Ethiopian Standards

The Ethiopian Standards are developed by national technical committees which are composed of different stakeholders consisting of educational Institutions, research institutes, government organizations, certification, inspection, and testing organizations, regulatory bodies, consumer association etc. The requirements and/ or recommendations contained in Ethiopian Standards are consensus based that reflects the interest of the TC representatives and also of comments received from the public and other sources. Ethiopian Standards are approved by the National Standardization Council and are kept under continuous review after publication and updated regularly to take account of latest scientific and technological changes.
Orders for all Ethiopian Standards, International Standard and ASTM standards, including electronic versions, should be addressed to the Documentation and Publication Team at the Head office and Branch (Liaisons) offices. A catalogue of Ethiopian Standards is also available freely and can be accessed in from our website.

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International Involvement
ESA, representing Ethiopia, is a member of the International Organization for Standardization (ISO), and Codex Alimentarius Commission (CODEX). It also maintains close working relations with the international Electro-technical Commission (IEC) and American Society for Testing and Materials (ASTM).It is a founding member of the African Regional Organization for standardization (ARSO).

More Information?
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